

## **Gonadotropin Releasing Hormone (GnRH) Agonist Test in Disorders of Puberty**

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## **GnRH Agonist Test in Disorders of Puberty. Outline.**

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- **Overview**
- **The nature of the problem**
- **Background endocrinology**
- **Background of this study: antecedent studies**
- **Adverse events of leuprolide**
- **Protocol #13472A**
- **Summary**

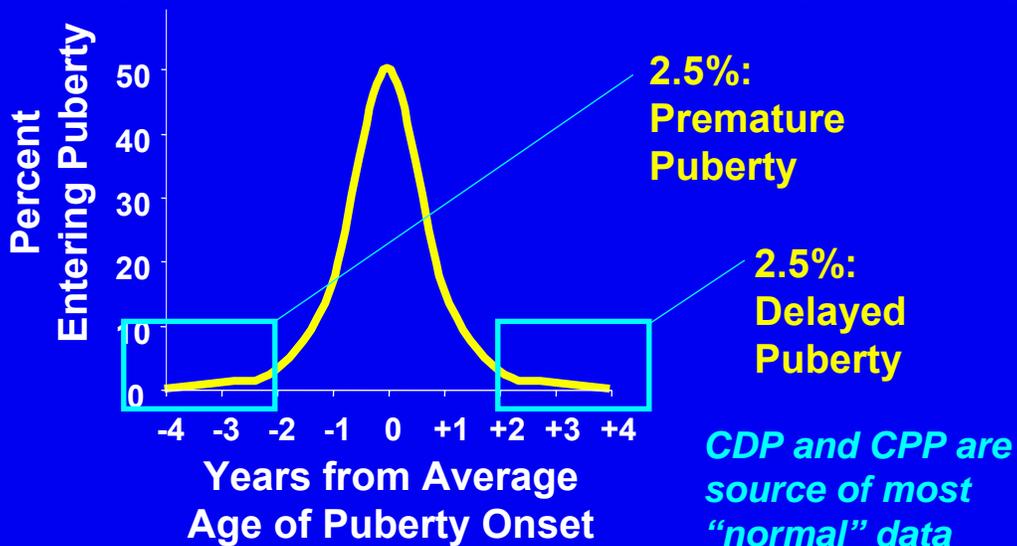
## Overview:

### GnRH Agonists are Promising Diagnostics

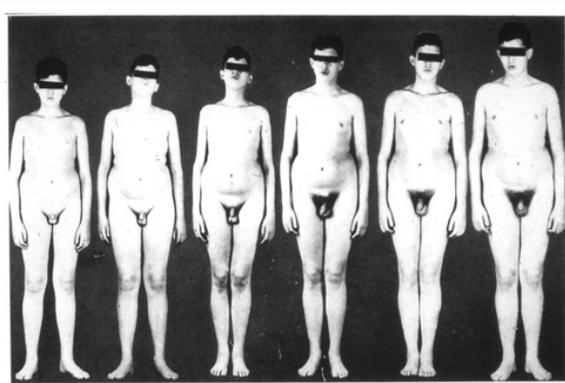
- Delayed Puberty (esp. a diagnostic problem in boys)
  - Constitutional Delay of Puberty (CDP, “an extreme variant of normal”) vs
  - Gonadotropin Deficiency (GnD)
- Premature (Precocious) Puberty (esp. in girls)
  - Idiopathic True / Central Precocious Puberty (CPP, “an extreme variant of normal”) vs
  - Normal Prepubertal *and*
  - Premature Pseudo-Puberty (diverse types)
- Need for Normative Data on Healthy Prepubertal & Early Pubertal Children

### CDP & Idiopathic CPP: “Extreme Variants of Normal”

#### Conceptual Definition of Premature & Delayed Puberty



## Problem 1: Differentiating Constitutional Delay of Puberty (CDP) from Gonadotropin Deficiency (GnD)



17.3 18.0 18.7 19.5 20.3 21.8  
Longitudinal F/U of Boy with CDP (years)

Wilkins L: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Thomas, Springfield, 1968.

- Delay mostly in boys
- CDP boys develop increasingly poor self-image after 14 years
- “Grow out of it”
- Cause: “nl variant”
- W/U: minimal
- Rx: reassurance  $\pm$  6 month T boost
- Contrasts with GnD

## Problem 2: Differentiating Idiopathic CPP from Normal Variants & Other Pseudo-Precocity. I.



- Precocity predominantly in girls
- CPP scary for child & parents
  - » moody
  - » periods?
  - » early growth arrest
- Over > 90% “nl stage just early”
- W/U: minimal (brain MRI when rapidly progressive)
- Rx: reassurance  $\pm$  GnRH ag chronically until ~11 yo
- Pseudo-precocity may be normal variant or due to neoplasm, etc

## Problem 2: Differentiating Idiopathic CPP from Normal Variants. II. The Problem of Early Thelarche

“Yesterday’s Precocious Puberty is Norm Today”

- N Y Times, December 7, 1999

“Doubters Fault Theory Finding Earlier Puberty”

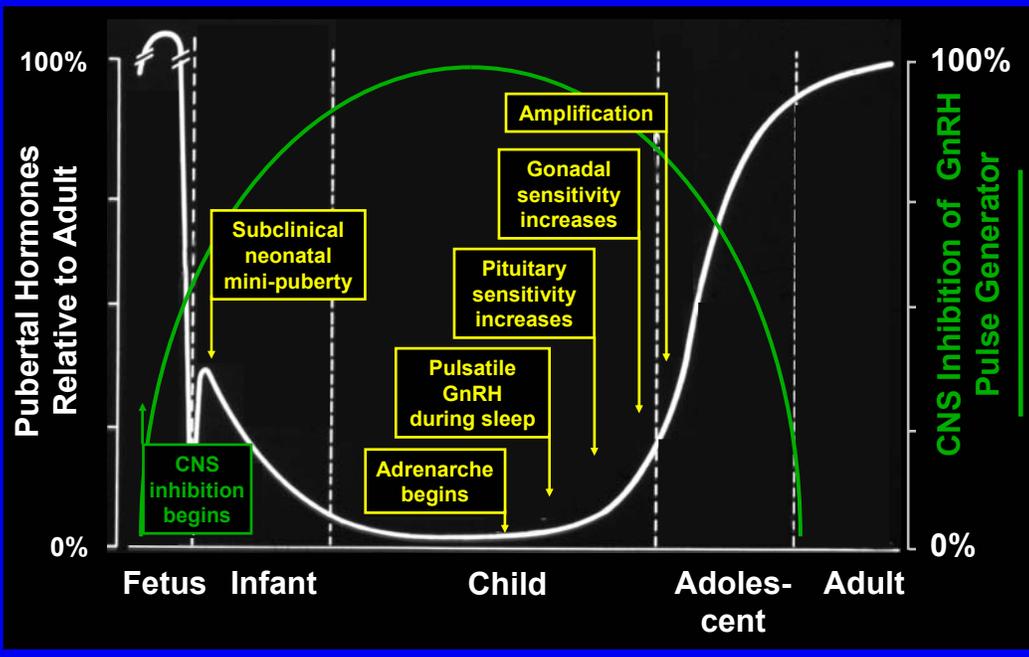
- N Y Times, February 20, 2001

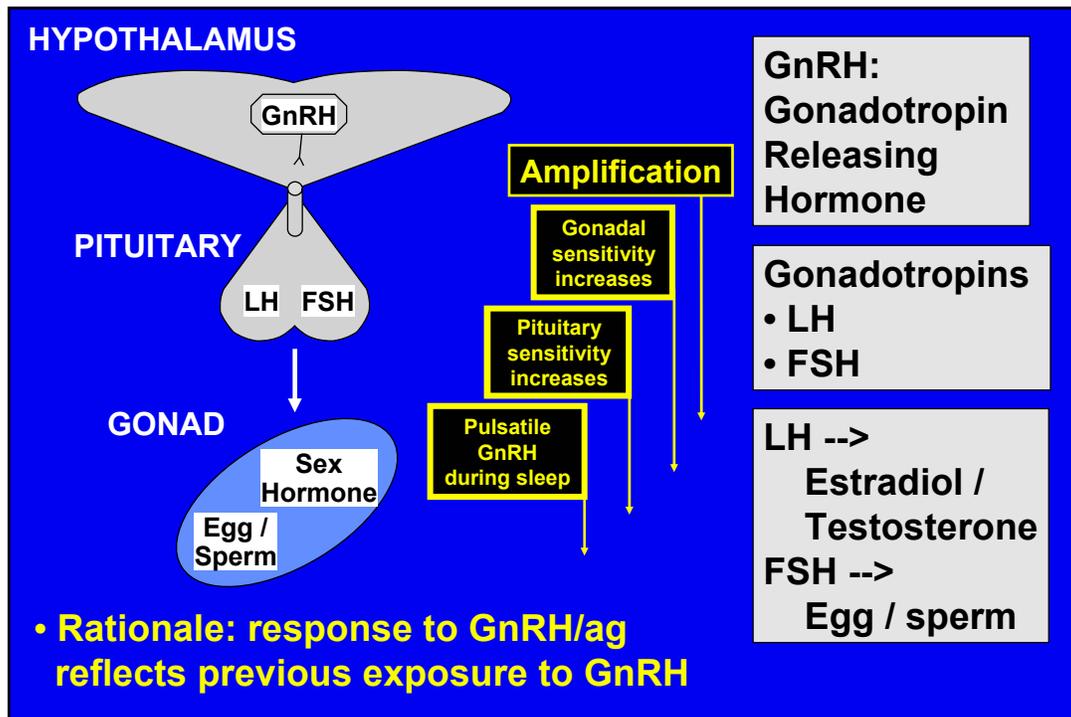
“2 Endocrinology Groups Raise Doubt on Earlier Onset of Girls' Puberty”

- N Y Times, March 3, 2001

- *While there is some evidence that breast development may be occurring 1-2 years earlier, esp. in the obese, age of menarche is unchanged--is this true puberty?*

## BACKGROUND ENDOCRINOLOGY OF PUBERTY





## BACKGROUND OF GnRH AGONIST TESTING. I.

- 1977: Nobel Prize for Discovery of GnRH
- '80's: Desensitizing Effect of Chronic GnRH Agonist Analogs -> Chronic GnRH Agonist Rx for CPP (ODP)
- 1985: PI GCRC Studies Under Expanded Syntex IND for Nafarelin Treatment of Central Precocious Puberty
- Examined patients' hormonal responses to 1st dose of nafarelin (out of my interest in the acute response being a potentially useful diagnostic test)

### Endogenous GnRH (Factrel®)



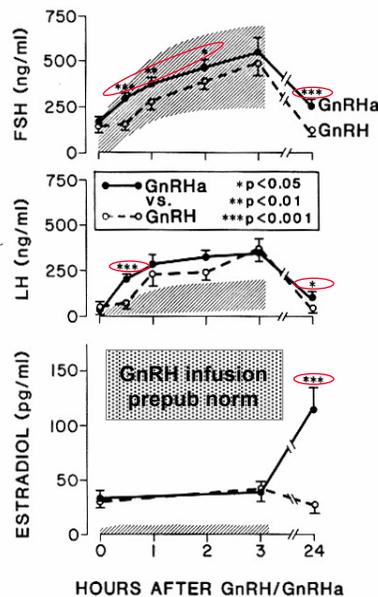
### Nafarelin (Synarel®)



### Leuprolide acetate (Lupron®, generic)



- **Subjects:**  
CPP girls starting nafarelin (GnRHa) Rx
- **Compared GnRH**  
3 hr infusion test to a nafarelin test of pituitary-gonadal axis
- **Results: LH & FSH responses to GnRHa greater & more prolonged than to GnRH --> estradiol response**



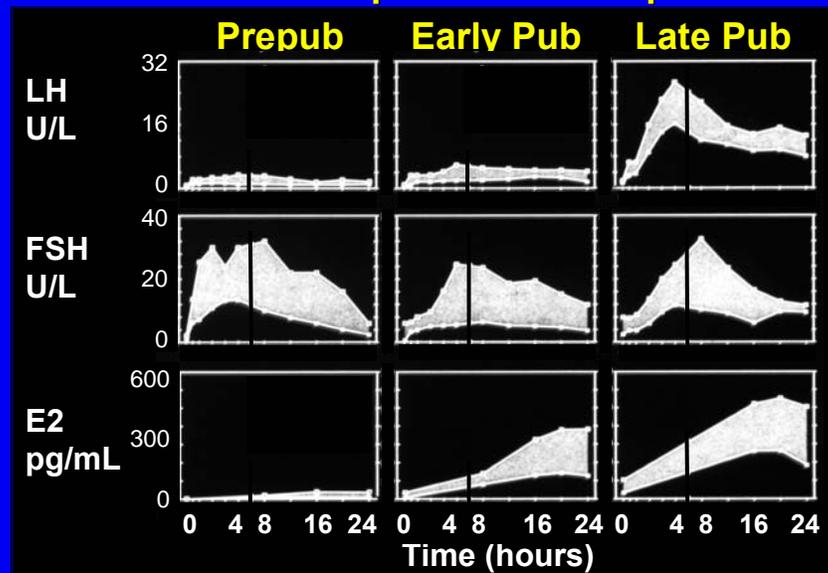
Rosenfield, et al JCEM 1986

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- 1985: PI GCRC Studies Under Expanded Syntex IND for Nafarelin Treatment of Central Precocious Puberty
  - Comparison of CPP's hormonal response to nafarelin dose #1 by injection to natural GnRH infusion test
    - » showed acute agonistic effect on LH-FSH-E2
- 13472A precursor protocols were pilot studies to explore diagnostic potential of nafarelin in children with known or suspected disorders of puberty (mostly CDP or CPP)

### Girls with variations of normal pubertal development

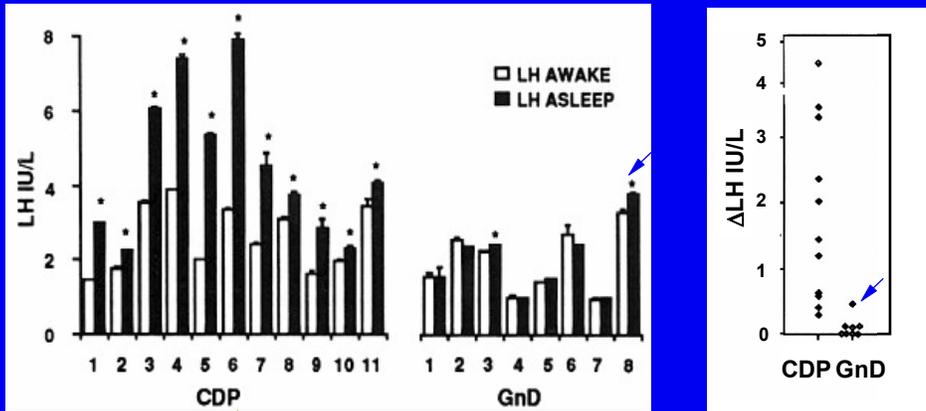
Nafarelin tests in girls at various pubertal stage



Goodpasture, et al. Clin Obstet Gyn 1993

**BACKGROUND: GnD vs CDP in prepubertal boys**

**Sleep Tests:**

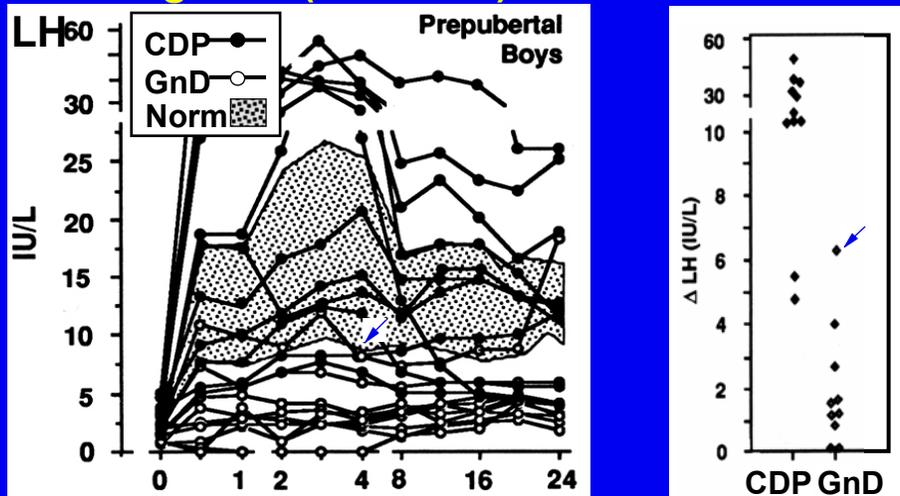


**Provisional sleep test discriminatory criterion:  $\Delta LH \geq 0.35$  U/L separates 18/19**

*Ghai, et al JCEM 1995*

**BACKGROUND: GnD vs CDP in prepubertal boys**

**GnRH Agonist (Nafarelin) Tests:**



**$\Delta LH \geq 4.8$  U/L discriminates 19/20**

*Ghai, et al JCEM 1995*

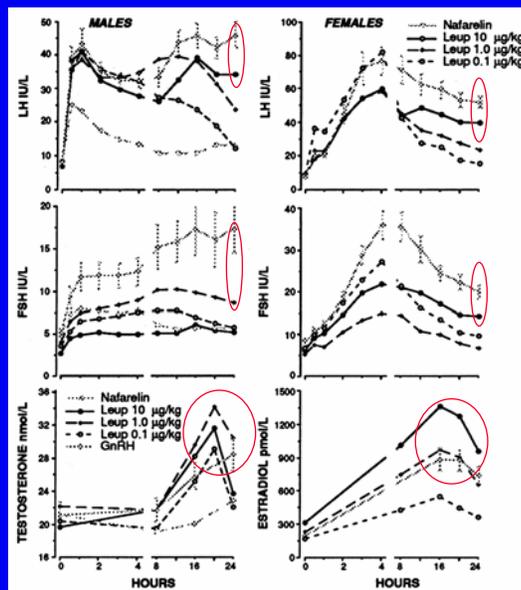
## BACKGROUND OF GnRH AGONIST TESTING. II.

- 1992-93: Syntex sold out & Searle not interested  
PI obtained IND #40,387 (1992): '93 nafarelin -> leuprolide
- Several GCRC protocols with co-investigators
  - » Hyperandrogenism in adult women and children
  - » Disorders of puberty (CDP, CPP, etc)
  - » TAP 1 yr bridge funding --> no further support
- FD-R-001012 (1994, ODP)
  - » Adult dose-response study & comparison to naf/GnRH
  - » GnD vs CDP
- FD-R-001473 (1997, OPD)
  - » Adult GnD trial of intermittent GnRHag Rx
- IND #60,003 (2000): leuprolide
- RO1-HD-39267 ('01): hyperandrogenism (child & adult)

**Dose-response study  
of leuprolide  
(Lupron®) in adults  
+  
comparison to GnRH  
(Factrel®) in men &  
historical nafarelin  
data**

### Results

- Lupron 10 mcg/kg similar to naf in LH & sex steroid stimulation
- Lupron less potent FSH stimulant



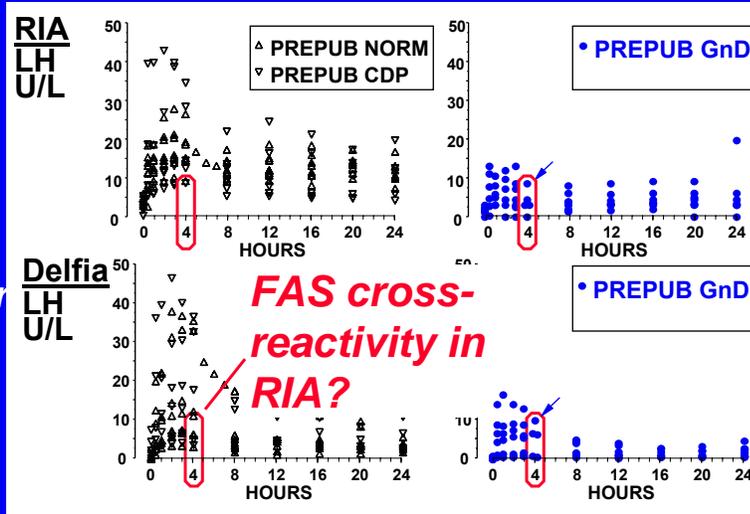
--Rosenfield, et al JCEM 1996

## Snags!

### 1. RIA results unlike monoclonal ("3rd gen.") assay for LH

#### Why?

- Microheterogeneity of LH
- Our RIA had enhanced specificity for bioactive LH, but incomp.
- Delfia  $\beta$ -subunit specific

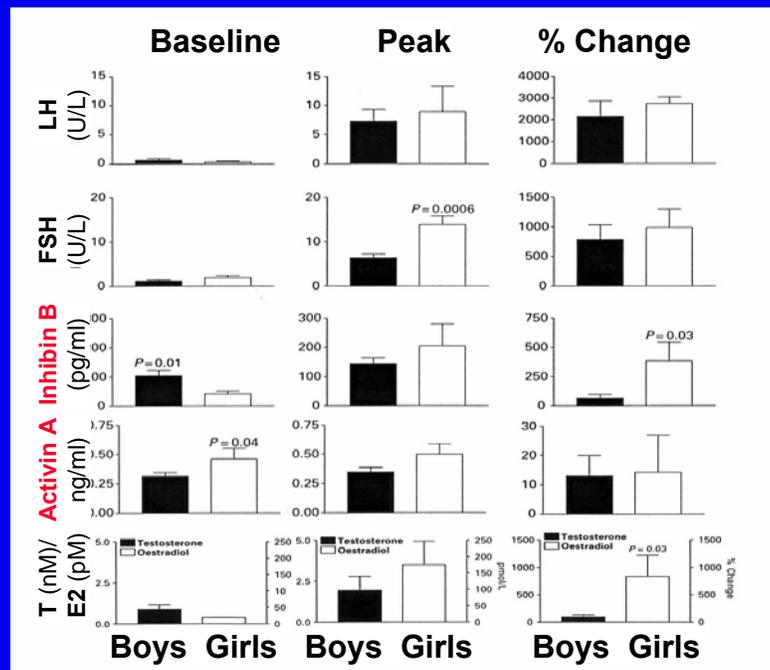


### 2. Alarmed freezer failure: lost samples before 2001

Meanwhile--  
other sex-specific  
potential  
end-points  
discovered  
in pubertal  
variants

Boys (n=11)  
& girls (n=7)  
w. BA >7.8 y

-- Elsholz, et al  
Hum Reprod  
2004



## **BACKGROUND OF GnRH AGONIST TESTING. III.**

### **Summary to Date.**

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- **Leuprolide not quite same as nafarelin re FSH stim**
- **Can't go back to discriminatory RIA**
- **Considerable promising preliminary data in children from multiple peer-reviewed studies at many levels (GCRC/site visits, FDA- and R01-reviews)**
- **Starting over**

## **ADVERSE EVENTS OF LEUPROLIDE TEST (1 Injx)**

### **Leuprolide tests in 577 adults & children: U of C 1993-2005**

- **No Serious Adverse Events**
- **Anticipated Side Effects**
  - **Children under 18 years of age (n = 332)**
    - » **IV-related: 3 (soreness, hematoma) -> one withdrew**
    - » **local allergic reaction (rash), transient: 1**
  - **Adults (n = 245)**
    - » **IV-related: 1**
    - » **local allergic reaction (rash), transient: 1**
    - » **Hormone-related side effects (post-study): 14**
      - **menstrual pattern change: 3 (1 pre-existing PCOS)**
      - **PMS symptoms mood, cramps, h/a: 11**  
(1 of the 3 *males* improved)

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      - menstrual pattern change: 3 (1 pre-existing PCOS)
      - PMS symptoms mood, cramps, h/a: 11  
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## ADVERSE EVENTS OF LEUPROLIDE TREATMENT

### Wide use of leuprolide long-term Rx (Depot Lupron®)

- Children with CPP (short stature)
- Adult men with prostate cancer
- (Adult women with endometriosis, fibroids, fertility Rx)

### Side effects of long-term Rx

- Depot -> 5-10% develop sterile abscesses at injx sites
- Hormone-related side effects
  - » Menstrual irregularity
  - » PMS symptoms (mood changes, swelling, h/a, etc)
  - » Memory effects? (data contradictory--Yaffe, JAMA 2003)
  - » osteopenia
- No increase in birth defects (*Jannsens 2000*)

## ADVERSE EVENTS OF LEUPROLIDE ACETATE. Response to Adverse Public Comment (4 letters)

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### Mis-informed and/or related to long-term therapy

- Package insert adverse effects are those of 2 yr Rx of prostate ca
- No “black box” warning
- Human evidence for adverse effect on autoimmunity insufficient to warrant warning
- Not a hazardous drug requiring chemo precautions

## Other Public Comments

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- Lawson Wilkins Pediatric Endocrine Society, The Endocrine Society, The American Society for Reproductive Medicine are unconcerned about leuprolide acetate test toxicity
- LWPES notes that “leuprolide is used in the routine diagnostic testing of children to determine the initiation of puberty...highly useful...normative data are sparse”
- The Endocrine Society *adds* that, while determining sleep-related LH secretion is the “gold standard,” it is (potentially) “less invasive than the leuprolide test.”

## PROTOCOL 13472A:

### GnRH Agonist Test in Disorders of Puberty

#### Hypothesis

Hormonal responses to GnRH agonist (GnRHag) test will distinguish among disorders of puberty as well as a sleep test.

#### Specific Aims

1. Distinguish among the causes of premature puberty:
  - a. idiopathic CPP (vs. *prem. thelarche*) vs healthy vols
  - b. gonadotropin-indep precocity (e.g., tumor) vs idiopathic CPP
2. Distinguish among the causes of delayed puberty:
  - a. GnD vs CDP

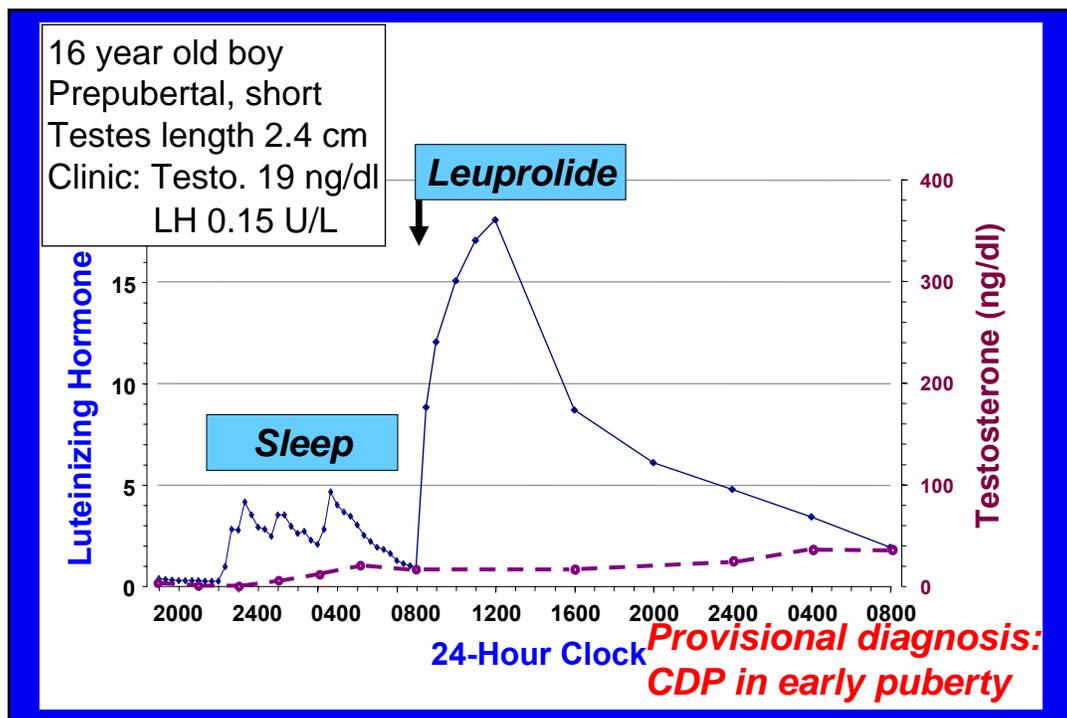
### CDP & Idiopathic CPP: "Extreme Variants of Normal"?

- **Practice assumes that these are normal variants**
  - Pubertal tempo, menstrual cyclicity, and fertility in adult life typically within broad range of normal
  - Familial in about half
- **Evidence that a small percent of these "normal variants" may *not* be normal:**
  - Slow tempo of those starting puberty at 6-7 years
  - Family history of delayed puberty in ~10-15% of GnD
  - GnRH receptor SNPs nominally associated with variations in timing of puberty (*Sedlmeyer, JCEM Oct '05*)
  - Mouse chromosomes 6 and 11 harbor genes that regulate pubertal timing (*Krewson, Endocrinol 2004*)
- **Normal population data needed to avoid misclassif.**

## PROTOCOL #13472A: GnRH Agonist Test in Disorders of Puberty

### Study Design

- Subjects: 20 per group of each sex.
  - » Normal volunteers:
    - Prepubertal male, 9-13 years old
    - Prepubertal female, 8-12 years old
    - Early pubertal male, 9-15 years old
    - Early pubertal female, premenarcheal, 9-15 years old
  - » Patient groups
    - CDP vs GnD
    - CPP vs gonadotropin-independent precocity and premature thelarche



## GnRH Agonist Test in Disorders of Puberty

### Analysis of Data. I.

Sleep test: significant increase LH  $\geq 0.35$  U/L provisionally defines puberty onset

- Normal range set at 5-95 %ile healthy volunteers
  - » Secondary: 5-95%ile for CDP (boys) & CPP (girls)

### GnRH agonist (leuprolide) test:

- Hormone response primary variables (group-specific)
  - » Pituitary: LH, free alpha subunit (FAS)
  - » Gonads: Sex steroid (T or E2), inhibin-B
- Sex- and stage-specific 5-95%ile ranges set for:
  - » Normals (healthy volunteers)
  - » CDP and CPP

## GnRH Agonist Test in Disorders of Puberty

### Analysis of Data. II.

- Boys (*common dx problem is delayed puberty*):
    - » Primary comparison: GnD vs CDP (stage-specific)
    - » Secondary: GnD vs healthy volunteers *and* CDP vs healthy volunteers
    - » *Tertiary: girls\**
  - Girls (*common dx problem is premature puberty*):
    - » Primary: CPP vs prepubertal healthy volunteers
    - » Secondary: CPP vs pseudo-pubertal groups\* (gonadotropin-independent precocity and premature thelarche)
    - » *Tertiary: boys\**
- \* *Power is limited for some sub-groups*

## **GnRH Agonist Test in Disorders of Puberty: Summary**

- **GnRH agonist testing is of minimal risk**
- **Study design straight-forward: normal vs abnormal**
- **Adequate statistical power for primary comparisons**
- **Significance for clinical care: great**
  - » This protocol will develop badly needed data on the hormonal responses to leuprolide in normal prepubertal and pubertal children, using commercially available state-of-the-art assays
  - » It will also provide data on the diagnostic value of the test for the most common pubertal disorders